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Effects of Selected Drugs on Spontaneously Occurring Abnormal Behavior in Beagles

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IORIO, L C , N EISENSTEIN, P E BRODY AND A BARNETT *Effects of selected drugs on spontaneously occurring abnormal behavior in beagles* PHARMACOL BIOCHEM BEHAV 18(3) 379–382, 1983 —A sub-population of beagles with abnormal behavioral patterns has been identified, isolated and tested for responsivity to selected classes of psychoactive drugs The abnormal behavior was ameliorated by anxiolytics and antidepressants but not by antipsychotics, an antihistaminic, an alpha-adrenergic blocker, a beta-adrenergic blocker, or an anticholinergic drug Improvement occurred after a single dose of the anxiolytic drugs but did not occur until 10–18 days after daily dosing with standard tricylic antidepressants and the MAO inhibitor isocarboxazid This delayed onset in beagles resembles that seen on use of these drugs in humans The results with these drugs suggest that the abnormal behaviors of the beagles are related to anxiety and are in part depressive in nature This colony provides an animal model of abnormal behavior which allows evaluation of the anxiolytic and antidepressant effects of drugs and estimates of the onset of action of antidepressant drugs

Anxiolytics Antidepressants Psychoactive drugs Abnormal behavior Dogs

THE evaluation of central nervous system (CNS) drugs in animals is based primarily on the drug's ability to ameliorate stimulus-altered behavior of animals For example, anxiety-like behaviors are induced by placing animals in conflict situations [3,11] and depressive behaviors can be evoked by subjecting animals to isolation [9,10], repeated no-win competitive situations [5], unavoidable shock environment [6], etc One exception may be the specially bred nervous dogs of Murphree and his collaborators [7,8] which appear to be susceptible to the tranquilizing effects of chlorpromazine [2] This report presents a description of a selected group of beagles with spontaneously occurring abnormal behaviors which are ameliorated by acute administration of standard anxiolytics and by repeated administration of standard antidepressant drugs

METHOD

Source of Beagles with Unusual Behavior

The beagles described in these studies were found in breeding colonies at the toxicology laboratories of Schering Corporation at Lafayette, NJ Attention was first called to them when investigators described some dogs (about 2% of the breeding colony) as "withdrawn," "depressed" and easy to handle compared to beagles with normal behavior These observations led us to isolate the dogs and test them for reactivity to various classes of psychoactive drugs

All beagles in the breeding colony were raised in a conventional fashion Litters are weaned at six weeks of age and housed in groups of 20–40 in large outdoor pens connected to inner rooms At twelve months of age the "depressed" beagles were identified on the basis of gross behavioral abnormalities (e g , lack of barking, reduced approach behavior to humans, etc), removed from the colony, and housed individually in standard dog cages (73 7×81 3×96 5 cm) Food and water were available ad lib and the animals were housed indoors with a 12 hour light/dark cycle

Quantitation of Behavior

The beagles selected from the breeding colony generally continued to show marked deviations from normal behavior exhibited by other beagles housed under similar conditions Unlike normal beagles, these beagles did not manifest postural/motor behaviors such as high levels of motor activity, exploration, alertness or barking and showed deficits in approach/attentional behavior such as not looking at and making eye contact with observer, not approaching and sniffing new objects, and not eating food on presentation Preliminary experiments also indicated that these dogs manifested decreased sexual activity when presented with estrous females

Based on these observations, a rating scale was devised to quantitate behavior before and after drug administration For simplification, behavior of dogs on presentation of food or in the presence of estrous females was excluded from this quantitation For other behaviors, a modification of the method of Irwin [4] was used, which involved scoring of 17 behavioral measures, 13 of which are associated with normal behavior of beagles in the presence of an observer and were given a score of 1 (one) when observed head above shoulders, tail horizontal or higher, dog located at front of cage,

TABLE 1

EFFECTS OF CHLORDIAZEPOXIDE DIAZEPAM AND AMOBARBITAL ON ABNORMAL BEHAVIOR OF BEAGLES

Drug	Daily Oral Dose* mg/kg	Number of Dogs Treated	Improved†‡	Percent Improved	Days of Treatment	Mean % MPI§
Chlordiazepoxide	5	8	5	63	3	65
	2 5	3	3	100	11	61
Diazepam	2 5	4	4	100	7	66
Amobarbital	10	4	4	100	2	52

*Doses used were based on data in literature

†Defined as number of dogs that exhibited scores ≥30% MPI (percent maximum possible improvement)

‡Improvement occurred on each treatment day (including the first day) without evidence of tolerance, i e magnitude and duration of improvement (4–6 hours) were similar on each day

§Mean percent maximum improvement see Method section for calculation

dog stands, sits, has medium-high spontaneous motor activity, barks at observer, looks at observer, allows eye contact, follows observer with eyes, approaches observer with cage door closed, or cage door open, and sniffs or nuzzles hand of observer The remaining 4 measures are not part of normal behavior of beagles and their absence was scored as 1 (one) withdraws to back of cage when cage door is opened, trembles, flinches when touched, or manifests increased muscle tension Of a maximum score of 17 on this rating scale, normal beagles consistently scored 16–17

Almost half of the pre-selected beagles (35 of 75) improved spontaneously within two weeks after individual housing, i e , began registering scores of 10 or higher, and were not used further in these experiments It is thought that the increased handling and attention in the experimental laboratory situation may have led to this improvement The remaining 40 abnormal beagles consistently scored 2–8 with a mean of 5 In two separate studies conducted to evaluate interrater reliability of scoring, Pearson r correlations were found to be 0 88 and 0 91

Drug Treatments

All studies were done in an open design For each dog, a mean baseline score was calculated from behavioral scores obtained 60–90 minutes after treatment on 4 consecutive days on which a gelatin capsule containing lactose (placebo) was given orally All dosing and behavioral evaluations were done in the mornings of test days Acute effects of test drugs were studied on day 1 at hourly intervals for up to 6 hours After day 1, dogs were evaluated prior to and 60–90 minute after drug administration each morning In each case the mean baseline score was then subtracted from the maximum possible score [17] to obtain the maximum possible range for improvement for each dog Scores obtained on each subsequent treatment day(s) with placebo or drug were converted to percent of maximum possible improvement (% MPI) This conversion allows a common standard for drug effects to be established in different animals, regardless of baseline score A drug was considered to be active on any day that the % MPI was ≥30% This is a stringent criterion based on data from many abnormal beagles showing that such high values were clearly outside the 95% confidence limits of baseline socres When an estimate of onset time for behavioral improvement was made in repeated dose studies, this was taken to be the 2nd day of the first 3 consecutive days on which % MPI was ≥30%

Because of the relatively small colony of beagles and the lengthy time-course of these experiments, testing of most drugs was limited to one or two doses The doses used were, as determined in pilot studies with normal beagles, the highest doses tolerated without overt adverse effects

RESULTS

Benzodiazepines and Amobarbital

Of the many drugs tested in beagles, only the benzodiazepine anxiolytics diazepam and chlordiazepoxide and the barbiturate amobarbital caused behavioral improvement on acute administration (Table 1) The effects occurred within one hour after the first treatment and lasted for 4–6 hours, after which abnormal behavior returned to baseline levels Similar activity without diminution was seen on repeated treatment with these drugs, e g , diazepam at 2 5 mg/kg PO had a similar time course of action on each of 7 consecutive days The anxiolytic properties of these drugs suggest that fear or anxiety may be a significant component of the abnormal behaviors of beagles Meprobamate (Table 3) was not active in these dogs at a relatively high doses for two days, but this treatment schedule may not have been sufficient to demonstrate the actions of this mild anxiolytic

Antidepressants

The two tricyclic antidepressants most commonly used in humans imipramine and amitriptyline, and the monoamine oxidase inhibitor isocarboxazid produced a partial improvement in abnormal behavior in about 50% of treated beagles with an onset time of about 2 weeks, improvement was never seen earlier (Table 2) In contrast, placebo-treated beagles never showed improvement Additional evidence that the observed improvements were drug-related includes the observations that once seen in any beagle, improvement was maintained at a relatively constant level by repeated drug administration and that behavior returned to pretreatment levels when treatment stopped (characteristic data with one dog shown in Fig 1), only one beagle throughout the history of our colony of 40 beagles, observed over 4 years, failed to return to its pretreatment abnormal behavior

Except for nomifensine and trazodone, the other antidepressants used for or being tested in human depression had

TABLE 2

EFFECTS OF ANTIDEPRESSANTS ON ABNORMAL BEHAVIOR OF BEAGLES

Drug	Daily Oral Dose, mg/kg	Treatment Days	Number of Dogs		Percent Improved	Latency to Improvement†, days	Mean % MPI‡
			Treated	Improved*			
	—	21	12	0	0	—	—
Imipramine	10	21	28	14	50	14	42
	5	21	12	2	17	12	29
Amitriptyline	15	21	6	3	50	10	43
	10	21	5	2	40	10	32
Protriptyline	10	21	4	1	25	13	56
	5	21	4	2	50	8	32
Isocarboxazid	10	21	4	4	100	17	59
Iprindole	15	15	4	1	25	8	20
	10	21	4	2	50	8	22
Doxepin	20	21	4	0	0	—	—
	10	22	4	1	25	2	36
Mianserin	10	21	4	1	25	15	32
Buproprion	10	21	4	1	25	17	34
Nomifensine	10	21	4	0	0	—	—
Trazodone	10	12	4	0	0	—	—
Viloxyzine	15	13	4	1	25	7	29

*Number of dogs that exhibited scores ≥30% MPI on 3 consecutive days Evaluations made 90 minutes after treatment are presented here Evaluations made 90 minutes before treatments were similar

†Defined as the 2nd day of 3 consecutive days on which scores were ≥30% MPI

‡Mean percent maximum possible improvement calculated by averaging scores on the 3 consecutive days of improvement from onset of improvement to the end of treatment remained relatively constant, i e , in no case was the level of improvement on any one day significantly greater than the mean % MPI reported here and only with iprindole did the improvement level decrease (% MPI remained above 30% for 4–5 days, then decreased gradually to <15% during the next 3–4 days)

activity in at least one abnormal beagle It seems likely that these observed effects are drug-related because, as stated above, behavioral improvement rarely occurs spontaneously or in placebo-treated beagles However, the low frequency of improvement and the small sample sizes preclude a more definitive interpretation of these data As indicated previously, higher doses of these drugs could not be used

Miscellaneous Drugs

A variety of drugs were found to be inactive in ameliorating abnormal behavior in beagles after either single or repeated doses (Table 3) These include stimulants such as d-amphetamine, methylphenidate, caffeine and cocaine, antipsychotics such as haloperidol, perphenazine, thioridazine and thiothixene, the antihistamine chlorpheniramine, the beta-adrenergic blocker propranolol, the alpha-adrenergic blocker phentolamine, and the anticholinergic scopolamine Stimulants such as d-amphetamine, methylphenidate, caffeine and cocaine were also inactive, the low baseline scores seen in the beagles precluded the possibility of observing whether this class of drugs can decrease scores

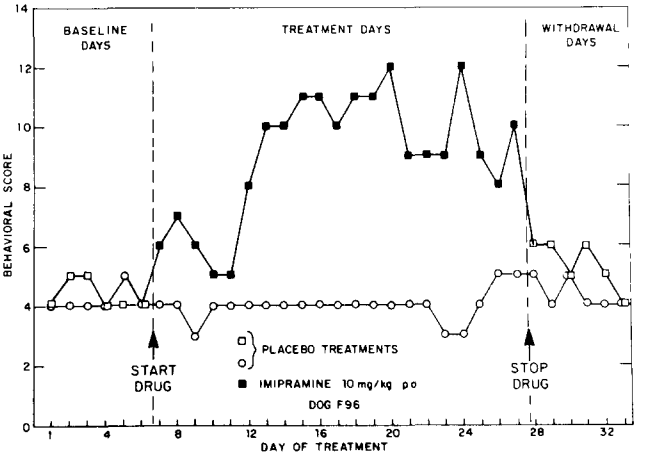

FIG 1 Effects of repeated daily treatment (21 days) of imipramine at 10 mg/kg PO to beagle F96 For this experiment, the onset time (2nd of 3 consecutive days on which % MPI was ≥30%) was day 13 During baseline and withdrawal days placebo capsules were administered Note return to baseline behavior when drug administration was stopped

DISCUSSION

A sub-population of beagles with abnormal behavior patterns has been identified and isolated from our breeding colony The behavioral abnormalities include locomotor retardation, stooped posture, and lack of responsiveness and alertness

Tested for responsitivity to drugs, the abnormal behaviors in these beagles were ameliorated by two general classes of drugs, the anxiolytics and the antidepressants The effects of these drugs appear to be relatively specific since other types of compounds, including standard antipsychotics, an

TABLE 3
DRUGS FOUND TO BE INACTIVE IN ABNORMAL BEAGLES

Drug	Single Daily Dose* mg/kg	Days of Treatment
Anxiolytics		
Meprobamate	100	2
Stimulants		
d-Amphetamine	0 5 and 1 0	7
Methylphenidate	10	2
Caffeine	50	2
Cocaine	10	10
Antipsychotics		
Haloperidol	0 3	6
Perphenazine	0 5	21
Thioridazine	2 5	10
Thiothixine	2 5	7
Others		
Chlorpheniramine	5	2
Propranolol	10	2
Phentolamine	5	2
Scopolamine	1	1

*Doses used were selected from data in literature as doses just below those that cause side effects in dogs All drugs were tested in groups of four dogs except for perphenazine, scopolamine and propranolol which were tested in 3 dogs

antihistamine, an alpha-adrenergic blocker, a beta-adrenergic blocker and an anticholinergic were inactive

Drugs active in the abnormal dogs include those with anxiolytic properties such as the benzodiazepines, and amobarbital which, although not classified as an anxiolytic drug, does produce sedation These drugs are active when given the first time and have relatively short durations of action (4–6 hr) These results suggest that the behavioral abnormalities of beagles are due to anxiety, although except for increased muscle tone, the dogs lack other behaviors commonly associated with anxiety in dogs such as urination, defecation, fear-biting, or cowering

The second class of active drugs was the antidepressants, which were active in about 50% of treated subjects but only after repeated dosing (10–17 days for standard tricyclics and the MAO inhibitor isocarboxazid) However, in contrast to the anxiolytics, once behavioral improvement occurs, it is maintained throughout the 24-hour intervals between daily dosing This delayed onset of activity is similar to that seen in humans and is the first demonstration to our knowledge of their activity occurring only after several days in an animal behavior experiment A second similarity is the insensitivity of some abnormal beagles to drug treatment since about one third of depressed humans appears to be refractory to treatment with these drugs [1] Although a large number of studies could not be done to determine whether there was cross sensitivity of beagles to the various standard antidepressants, there appeared to be a tendency in that direction Of 8 beagles that received both imipramine and amitriptyline, 3 reacted to both drugs, 3 reacted to neither drug, one reacted to imipramine alone, and one reacted to amitriptyline alone, of the 4 beagles that reacted to isocarboxazid, 3 were also improved by imipramine

The data in this report support the view that the abnormal beagles represent an animal model of behavior that has several unique advantages over existing models First, the behavioral abnormalities occur spontaneously in a subpopulation of beagles which receives no special treatment such as isolation or subjection to learned-helplessness paradigms Second, the abnormal behaviors are chronic, some dogs have been in our testing colony for 4 years and the frequency of spontaneous remissions is low (<5%) Moreover, a drug-induced improvement returns to baseline levels within a few days after cessation of drug treatment Third, it represents a behavioral model that is responsive to only 2 classes of drugs, anxiolytics and antidepressants The possibility that the antidepressants possess anxiolytic properties, which appear only after repeated administration cannot be ruled out with certainty Irrespective of the mechanism it is clear that this animal model is susceptible to the pharmacologic activity of both anxiolytics and standard tricyclic antidepressants

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